

**Amendments to the Specification:**

On page 31, lines 3-10, please substitute the following paragraph:

The present invention also relates to *B. subtilis* strain-DSM4393, DSM17158, containing the *B. subtilis* plasmid pMIX91 (deposited at the DSMZ, Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany on 21<sup>st</sup> February 2005, SB202pMIX91) and to *B. subtilis* strain-4A423, DSM17159, containing the *B. subtilis* plasmid pMIX101 (deposited at the DSMZ, Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany on 21<sup>st</sup> February 2005, 1A423 pMIX101).

On page 31, line 22, through page 32, line 13, please substitute the following paragraph:

Another aspect of the present invention relates to a kit which can be used for conducting the inventive process for generating and detecting recombinant DNA sequences in a prokaryotic host cell. In a first embodiment the kit comprises at least a first container which comprises cells of the *E. coli* strain AB1157 as prokaryotic host cell, a second container which comprises cells of the *E. coli* strain AB1157 containing the *E. coli* plasmid pACYC184 or the *E. coli* plasmid pMIX100, which can be used as recipient DNA molecule, and a third container comprising cells of the *B. subtilis* strain-DSM4393, DSM17158, containing the *B. subtilis* plasmid pMIX91, or cells of the *B. subtilis* strain-4A423, DSM17159, containing the *B. subtilis* plasmid pMIX101, which can be used as donor DNA molecule. In a second embodiment of the invention the kit comprises at least a first container which comprises cells of the *E. coli* strain MXP1, which is a MutS<sup>-</sup> mutant of strain AB1157, as prokaryotic host cell, a second container which comprises cells of the *E. coli* strain AB1157 containing plasmid pACYC184 or pMIX100 and a third container comprising cells of the *B. subtilis* strain-DSM4393, DSM17158, containing the plasmid pMIX91, or cells of the *B. subtilis* strain-4A423, DSM17159, containing the plasmid pMIX101. In still another embodiment the kit comprises at least a first container which comprises either cells of the *E. coli* strain AB1157 or the *E. coli* strain MXP1, a second container comprising DNA

of the *E. coli* plasmid pACYC184 or pMIX100 and a third container comprising DNA of the *B. subtilis* plasmid pMIX91 or the *B. subtilis* plasmid pMIX101.

On page 35, lines 3-21, please substitute the following paragraph:

Figure 9 illustrates schematically the strategy for cloning the *oxa7*, *oxa11* and *oxa5* genes into the *E. coli* plasmid pMIX100 and into the *B. subtilis* plasmid pMIX101. For cloning *oxa7*, *oxa11*, and into pMIX100 the genes were amplified using primers containing either *Pst*I or *Xho*I at their 5' ends. After digestion with those enzymes, the amplified DNA fragments were independently ligated with pMIX100, which was previously cut with *Pst*I + *Xho*I. Competent cells of *E. coli* DHB10 were electroporated with the ligation mixtures, and colonies harboring positive clones were phenotypically selected by chloramphenicol-resistance/white color on LB plates containing Cm (30 µg/ml) + X-Gal (80 µg/ml) + IPTG (0.5 mM). Plasmid DNAs were obtained and analysed by restriction mapping. Results confirmed that pMIX104 carries *oxa7*, pMIX106b carries *oxa11* and pMIX107 carries *oxa5*. For cloning into the *B. subtilis* plasmid pMIX101 *oxa7* was obtained as a 0.9-kb fragment from pMIX104 by restriction with *Pst*I and *Xho*I and ligated with pMIX101, which was previously cut with the same enzymes. After transformation of *B. subtilis*-1A423\_DSM17159 competent cells with the ligation product, cells were selected in LB containing 0.5 µg/ml of erythromycin (Erm).

On page 38, Table 1, please substitute the following table:

Table 1: Bacterial strains

Strains	Genotype	Reference or source
<i>E. coli</i> AB1157 $\text{NaI}^R$ hsd	thr1 leu6 proA2 his4, thi1 argE3 lacY1 galK2 ara14 xyl15 mtl1 tsx33 str31 supE44thr <sup>+</sup> hsdR-B nal <sup>R</sup>	M. Radman strain collection
<i>E. coli</i> MIXP1	As AB1157 $\text{NaI}^R$ R <sup>-</sup> but mutS::Tn5 (kan <sup>R</sup> )	mutS allele from M. Radman strain collection
<i>E. coli</i> DH5 $\alpha$	SupE44 $\Delta$ lacU169 ( $\Phi$ 80lacZ $\Delta$ M15) hsdR17 recA1 endA1 gyrA96 thi-1 rel A1	(8)
<i>B. subtilis</i> <del>DSM4383</del> <u>DSM17158</u>	aroB2 trpC2 his6	German Strains collection DSMZ (Deutsche Sammlung von <del>Mikroorganismen</del> <u>Mikroorganismen</u> und Zellkulturen GmbH)

On page 43, lines 18-25, please substitute the following paragraph:

First, the *spec<sup>R</sup>* gene was obtained as a *Sac*I fragment of 1294 bps in length from the plaxmid pic156. The fragment was purified and then ligated to *Sac*I-digested pIL253. *B. subtilis* ~~DS4383~~DSM17158 competent cells were transformed with the ligation mixture and transformants were selected on LBA plates containing 75  $\mu\text{g ml}^{-1}$  of spectinomycin. Restriction analyses of plasmid DNA obtained from transformants confirmed that they harboured pILF253-derivatives carrying *spec<sup>R</sup>* gene.

On page 44, lines 1-11, please substitute the following paragraph:

In a second step, the *phleo*<sup>R</sup> gene was cloned into pIL253-spec. Plasmid pUC19-phleo was digested with *Eco*RI and *Sal*I restriction enzymes and the fragment of 574 bps in length corresponding to *phleo*<sup>R</sup> was gel-purified and ligated to pIL253-spec, previously digested with the same two enzymes. *B. subtilis* DSM4393DSM17158 competent cells were transformed with the ligation mixture and transformants were then selected on LBA plates containing 60 µg ml<sup>-1</sup> of phleomycin. Restriction analyses of plasmid DNA obtained from transformants demonstrated that they harboured the expected 6.69-kb plasmid, carrying both *spec*<sup>R</sup> and *phleo*<sup>R</sup> genes. Plasmid was named pMIX91 (see figure 3).

On page 45, lines 14-27, please substitute the following paragraph:

The four *oxa* genes were cloned into the *E. coli* plasmid pACYC184, whereas *oxa7*, *oxa11* and *oxa1* were cloned into the *B. subtilis* plasmid pMIX91 as well. The cloning of the genes was done as follows: *oxa7* was amplified by PCR with primers designed to introduce *Sca*I and *Ppu*MI sites at the 5' and 3' ends, respectively, of the amplified DNA (primers OLG1 with SEQ ID No. 1 and OLG2 with SEQ ID No. 2). The PCR product was digested with those restriction enzymes and the resulting fragment of 991 bps in length was ligated to pMIX91, previously cut with the same enzymes. *B. subtilis* DSM4393DSM17158 competent cells were transformed with the ligation mixture and transformants selection was done on LBA plates containing 75 µg ml<sup>-1</sup> of spectinomycin. Restriction analyses of plasmid DNA obtained from transformants demonstrated that they harboured the expected 6.69 kb plasmid pMIX94 carrying *oxa7* (see figure 3).

On page 46, line 27 through page 47, line 12, please substitute the following paragraph:

To clone *oxa11* and *oxa1* into pMIX91, genes were amplified by PCR using primers designed to introduce *ScaI* and *EcoO109I* sites at the 5' and 3' ends, respectively, of the amplified DNA. OLG9 (SEQ ID No. 9)/OLG10 (SEQ ID No. 10), and OL11 (SEQ ID No. 11)/OLG8 primers pairs were used to amplify *oxa11* and *oxa1* respectively. The PCR products were digested with *ScaI* and *EcoO109I* and the resulting fragment of 995 bps (*oxa11*) and 934 bps (*oxa1*) were independently ligated to pMIX91, previously cut with the same enzymes. *B. subtilis*-DSM4393 DSM17158 competent cells were transformed with the ligation mixture and transformants selection was done on LBA plates containing 75  $\mu\text{g ml}^{-1}$  of spectinomycin. Restriction analyses of plasmid DNA obtained from transformants demonstrated that they correspond to the expected plasmids pMIX98 (6.98kb) and pMIX99 (6.83kb). See figure 3.

On page 52, lines 5-9, please substitute the following paragraph:

The comparison of DNA and deduced amino acid sequences of recombinant genes revealed as well that 53% of them correspond to new *oxa* genes (see table[[ 4]]5). Since no frameshifts or stop codons were generated during recombination, they could putatively encode for 38 new functional  $\beta$ -lactamases.

On page 52, lines 10-12, please substitute the following table heading:

Table[[ 4]]5: Comparison of nucleotide and deduced amino acid sequences of recombinant *oxa* genes obtained by in vivo recombination

On page 53, lines 10-11, please substitute the following paragraph:

Bacterial strains and plasmids used in this example are shown in table[[ 5]]6 and table[[ 6]]7, respectively.

On page 54, please replace Table 5 with the following table:

Table[[ 5]]6: Bacterial strains:

Strains	Genotype	Reference or source
<i>E. coli</i> AB1157 $\text{NaI}^R$ $\text{hsd}^-$	<i>thr1 leu6 proA2 his4, thil argE3 lacY1 galK2 ara14 xyl15 mtl1 tsx33 str31 su-pE44thr<sup>+</sup> hsdR<sup>-</sup>B nal<sup>R</sup></i>	M. Radman strain collection
<i>E. coli</i> MIXP1	As AB1157 $\text{NaI}^R$ $\text{R}^-$ but <i>mutS::Tn5</i> ( $\text{kan}^R$ )	<i>mutS</i> allele from M. Radman strain collection
<i>E. coli</i> DH10B	$\text{F}^-$ <i>mcrA</i> $\Delta$ ( <i>mn<sup>-</sup> hsdRMS-mcrBC</i> ) $\Phi 80$ <i>lacZ</i> $\Delta$ m15 $\Delta$ <i>lacX74</i> <i>recA1 endA1 a-raD139</i> $\Delta$ ( <i>ara, leu</i> )7697 <i>galU galK</i> $\lambda^-$ <i>rpsL nupG</i>	Invitrogene Life Technologies Cat 18290-015
<i>B. subtilis</i> 4A423DSM17159	<i>argGH15 leuB8 recA4 thr-5 hsd<sub>m</sub> R<sup>+</sup>M<sup>+</sup></i>	Bacillus Genetic stock center (Ohio State University, USA)

On page 55, please substitute the following table heading:

Table[[ 6]]7: Plasmids

On page 57, lines 12-19, please substitute the following paragraph:

For cloning into the *B. subtilis* plasmid pMIX101 *oxa7* was obtained as a 0.9-kb fragment from pMIX 104 by restriction with *Pst*I and *Xho*I and ligated with pMIX101, which was previously cut with the same enzymes. After transformation of *B. subtilis*-4A423\_DSM17159 competent cells with the

ligation product, cells were selected in LB containing 0.5 µg/ml of erythromycin (Erm). Plasmid DNA was obtained from 24 transformants and analysed by restriction. Results confirmed that all clones contained the 7-kb plasmid pMIX105.

On page 58, lines 16-19, please substitute the following paragraph:

Results obtained are summarized in table[[ 7]]8. In the wild-type strain, recombinants were obtained in experiments using either identical or 5% divergent *oxa* genes, whereas in the MutS<sup>-</sup> mutant, recombination also happened between 22% divergent genes.

On page 59, please substitute the following table heading:

Table[[ 7]]8: In vivo recombination frequencies obtained between *oxa* genes in both wild-type and MutS<sup>-</sup> *E. coli* strains.